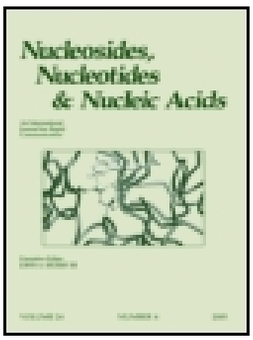

Nucleosides, Nucleotides and Nucleic Acids

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Synthesis of Pyridone-based Nucleoside Analogues as Substrates or Inhibitors of DNA Polymerases

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ABSTRACT
The synthesis and characterization of novel acyclic and cyclic pyridone-based nucleosides and nucleotides is described. In total, seven nucleosides and four nucleotides were synthesized. None of the tested nucleosides showed inhibitory properties against Klenow exo- polymerase and M.MuLV and HIV-1 reverse transcriptases. The nucleotides containing 4-chloro- and 4-bromo-2-pyridone as a nucleobase were accepted by the Klenow fragment, but at the expense of fidelity and extension efficiency.

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Pyridone nucleobase; nucleoside analogues; modified nucleotides; Klenow exo-; M.MuLV; HIV-1 RT

Introduction

Nucleoside analogues act as antitumor and antiviral agents via inhibition of various enzymes, including DNA and RNA polymerases.[1,2] Interest in acyclic nucleosides started in 1970 when acyclovir was reported as a potent anti-viral agent.[3,4] Nucleoside analogues have been in clinical use for almost 50 years and have become cornerstones of treatment for patients with cancer or viral infections.[5] Modified nucleotides also can be incorporated in nucleic acids in competition with their natural counterparts. The design of modified base pairs that can be replicated is of great demand for creation of new forms of xeno-DNA.[6] Thus, the synthesis and study of modified bases can reveal how broadly the structures can vary within the polymerase active site, outlining the future design parameters for man-made DNA-like systems. The first successful effort to expand the genetic code has been the identification of the isoC–isoG pair.[7] In 1994, Schweitzer and Kool introduced the concept of non-polar isosteres of natural DNA bases,[8] proposing the structures that have been the closest possible structural mimics of natural bases but lack hydrogen-bonding groups. It was shown that DNA polymerases could exert high fidelity even when a base pair completely lacks the polar Watson–Crick hydrogen bonds. Nucleoside derived from difluorotoluene, nonpolar nucleoside analogue but identical to

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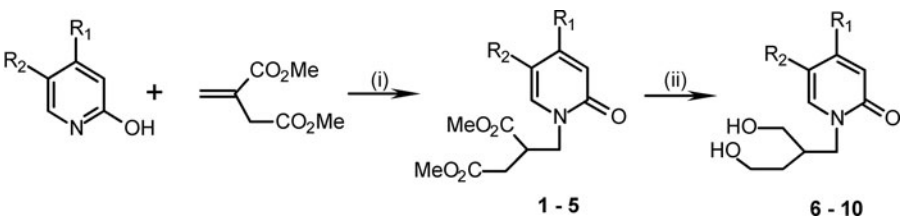

Figure 1. Synthesis of acyclic 2-pyridone nucleosides. Reagents and conditions: (i) DBU, DMF, rt, 24 h, 58–79%; (ii) $NaBH_4$, $CaCl_2$, THF, 0°C → rt, 18 h, 23–83%. **1, 6** $R_1 = H$, $R_2 = H$; **2, 7** $R_1 = OH$, $R_2 = H$; **3, 8** $R_1 = Cl$, $R_2 = H$; **4, 9** $R_1 = Br$, $R_2 = H$; **5, 10** $R_1 = H$, $R_2 = COOH$.

thymidine in size, shape, and conformation is placed within a DNA strand paired opposite adenine with high fidelity and efficiency.[9,10]

In this work we chose various pyridone bases as possible nucleobases for synthesis of various cyclic and acyclic modified nucleoside derivatives that could be used in biochemical research as enzymes inhibitors, and modified nucleotides as polymerase substrates for DNA biosynthesis.

Results and Discussion

Acyclic nucleosides were synthesized by the Michael addition of different heterocyclic bases (pyridone nucleobase) according to the synthetic method proposed by Guillarme et al.[11] In total, 20 heterocyclic bases with different substituents in 2-hydroxypyridine and 2-aminopyridine ring were screened as the Michael donors in the synthesis of acyclic nucleosides, and dimethyl itaconate was used as a Michael acceptor. The reaction mixtures were analyzed by high-performance liquid chromatography–mass spectrometry (HPLC-MS) and five 2-hydroxypyridine bases were selected for further synthesis of new acyclic nucleosides (Figure 1).

Synthesized compounds **1**–**5** were purified by reverse phase chromatography or silica gel column chromatography in a moderate yield. Reduction of diester adducts by $Ca(BH_4)_2$ afforded acyclic nucleosides **6**–**10** in a moderate to high yield. The NMR spectra of product **7** were consistent with those reported previously.[11]

The same heterocyclic pyridone bases were used for the synthesis of cyclic nucleosides. The nucleosides were synthesized according to the published procedures with modifications.[12–15]

Pyridone nucleobases were silylated using bis(trimethylsilyl)acetamide (BSA). After complete dissolution, silylated derivatives were glycosylated with 1,3,5-tri-*O*-acetyl-2′-deoxyribose (Figure 2). Tin (IV) tetrachloride was applied as a Lewis acid catalyst in the glycosylation reaction. The formed acetylated nucleosides were further purified by reversed phase chromatography or silica gel column chromatography. Compounds were isolated in high yields except compound **15** (yield 32%). Such a low yield could be due to poor solubility of 6-hydroxynicotinic acid. The deprotection step with 1 M sodium methylate solution in methanol and reversed phase chromatography purification afforded modified nucleosides **16**–**20** in good yields (63–73%).

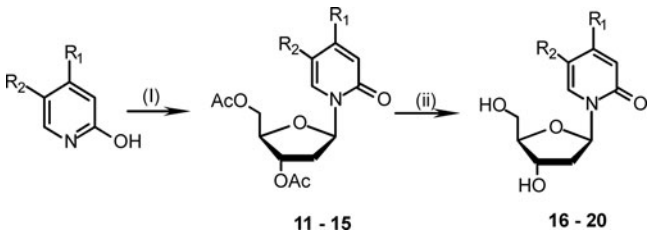

Figure 2. Synthesis of 2′-deoxynucleosides bearing pyridone-nucleobase. Reagents and conditions: (i) BSA, CH_3CN, rt, 40 min; then 1,3,5-tri-O-acetyl-D-ribose, $SnCl_4$, rt, 15 h, 32–97%; (ii) 1 M $NaOCH_3$, CH_3OH, rt, 20 min, 63–73%. **11**, **16** $R_1 = H$, $R_2 = H$; **12**, **17** $R_1 = OH$, $R_2 = H$; **13**, 18 $R_1 = Cl$, $R_2 = H$; **14**, **19** $R_1 = Br$, $R_2 = H$; **15**, **20** $R_1 = H$, $R_2 = COOH$.

Nucleotides **21**–**25** were prepared from nucleosides **16**–**20** according to the widely used one-pot method[12,16,17] with slight modifications (Figure 3). The lyophilized nucleosides were dissolved in dry triethyl phosphate and reacted with phosphorous oxychloride with addition of 1,8-bis(dimethylamino)naphthalene ("proton sponge"). The reactive intermediate phosphorochloridate was coupled with a mixture of tributylamine and tributylammonium diphosphate in dimethylformamide (DMF) to yield nucleotides **21**–**25**. The synthesized triphosphates were purified by anion exchange chromatography using diethylaminoethyl-(DEAE-) Sephadex A25. Nucleotides, as well as acyclic and cyclic nucleosides, were characterized by NMR spectroscopy and HPLC-MS analysis.

Novel 2-pyridone-based nucleosides as inhibitors of DNA biosynthesis

Acyclic and cyclic nucleoside analogues **6**–**10** and **16**–**20** were evaluated in the DNA biosynthesis assays. Representatives of two classes of DNA polymerases were employed, namely Klenow exo- (class A) and two reverse transcriptases – M.MuLV and HIV-1. In these assays, DNA primer was hybridized to the template strand to form fully complementary duplex bearing 3′ single-stranded region, facilitating primer extension using dTTP up to four consecutive events. The presence of compounds **6**–**10** and **16**–**20** (up to 10,000 fold excess over dTTP) in the reaction mix did not inhibit the incorporation of dTTP by all three polymerases tested (data not shown). Given the assay was highly sensitive (of single nucleotide level),

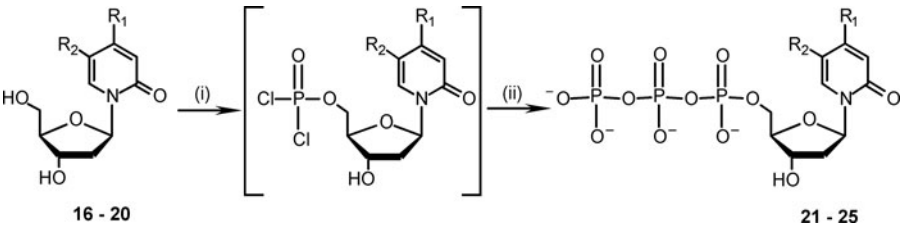

Figure 3. Synthesis of modified 2′-deoxynucleotides. Reagents and conditions: (i) proton sponge, $POCl_3$, triethyl phosphate, 0°C → rt, 80 min; (ii) tributylamine, tributylammonium pyrophosphate, DMF, rt, 7 min. **21** $R_1 = H$, $R_2 = H$; **22** $R_1 = OH$, $R_2 = H$; **23** $R_1 = Cl$, $R_2 = H$; **24** $R_1 = Br$, $R_2 = H$; **25** $R_1 = H$, $R_2 = COOH$.

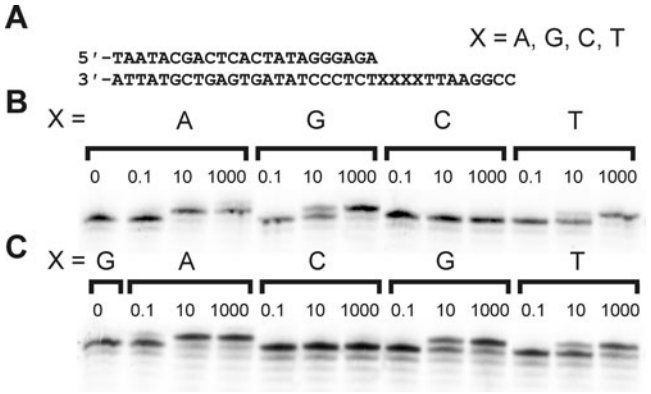

Figure 4. (A) Sequences of DNA template/primer duplexes used in this study. (**B**) and (**C**). Autoradiograms of 15% denaturing polyacrylamide gel electrophoretic analysis of products of DNA synthesis, using a ^{33}P-5′-end-labeled 23 nt primer as shown in part (**A**). Klenow exo- (50 nM) in Glu buffer in the presence of 5 nM DNA duplex, 1 mM Mg^{+2} and varying concentrations (0.1, 10, 1000 μM) of (B) **23** and (C) **24**. For further details, see sections Experimental and DNA Biosynthesis Assay.

the inhibitory power of the compounds analyzed is low. This, however, does not rule out the compatibility of compounds **6**–**10** and **16**–**20** with the active center of polymerases, requiring millimolar or higher concentrations of tested compounds to observe inhibitory effects.

DNA biosynthesis using 2-pyridone-based nucleotides

Compounds **21**–**25** were evaluated for acceptance by DNA polymerases in the DNA biosynthesis reaction. These compounds were used instead of natural deoxynucleoside triphosphates in an assay analogous to the one described above. Since nucleobase structures in all instances were clearly different from the natural pyrimidine-based moieties, DNA template strand was selected in a way to provide platform for incorporation of residue, complementary to any of natural nucleobases; therefore, four separate assay systems were used. Compounds **21**, **23**, and **24** were found to be accepted for incorporation into DNA structure by all three polymerases tested in a sequence-specific manner. While compounds **23** and **24** were readily accepted by Klenow exo- and to the lesser extent by reverse transcriptases M.MuLV and HIV-1, significantly lower yet observable incorporation was observed for compound **21** by all three respective enzymes. For all compounds incorporated, the following nucleobases on template strand were invariantly preferred: A≈G>T>>>C (Figure 4).

Of special note is the clearly expressed preference for all enzymes to extend DNA primer by single nucleotide only. Given that affinity of Klenow exo- toward compounds **23** and **24** is of the same order as for dTTP (not shown), one would expect that this reaction proceeds up to four events in a row, as determined by the structure of DNA duplex and happens in the case of dTTP. The observed single incorporation event witnesses structural obstacles for pyridone-based nucleotides toward further DNA extension. At the same time, high affinity for these triphosphates make

them nucleobase-based but not deoxyribose-based terminators of DNA biosynthesis. Benzene, naphthalene, pyridine, indole, and other *C*- and *N*-nucleoside analogues that formed stable duplexes where the self-pair was held together only by hydrophobic forces and stacking with the neighboring base-pairs have been developed previously.[18] Some of these self-pairs replicated by the Klenow fragment with reasonable efficiency and good fidelity. However, as in the case of the 4-chloro- (**23**) and 4-bromopyridone (**24**), the incorporation of the next dNTP did not work efficiently in any case. It was supposed that the inefficient extension of the chain was due to the lack of minor-groove interactions.[18] However, further research is needed to elucidate this phenomenon.

Conclusions

The present work describes the synthesis and characterization of seven novel acyclic and cyclic nucleoside derivatives containing 2-pyridone ring as a nucleobase. None of them showed inhibitory properties against Klenow exo- polymerase, M.MuLV, and HIV-1 reverse transcriptases. In addition, four 2-pyridone-based nucleotides were synthesized, and two of these, 4-chloro- and 4-bromo-2-pyridone nucleobases containing nucleotides, were rather efficiently incorporated into DNA by the Klenow fragment, but at the expense of fidelity and extension efficiency.

Experimental

All commercial chemicals and solvents were purchased from Sigma-Aldrich or Merck, and used without further purification. Thin layer chromatography (TLC) was carried out on 25 TLC aluminum sheets coated with silica gel 60 F_{254} (Merck) and column chromatography on silica gel 60 (0.04–0.063 nm) (Merck). Reverse phase chromatography was carried out on Grace flash cartridges C-18. NMR spectra were recorded in DMSO-d_6, $CDCl_3$, or D_2O on Bruker Ascend 400, ^{1}H NMR – 400 MHz, ^{13}C NMR – 100 MHz, and ^{31}P NMR – 162 MHz. Chemical shifts are reported in ppm relative to solvent resonance signal as an internal standard. HPLC-MS analyses were performed using a HPLC system equipped with a photo diode array detector (SPD-M20A) and a mass spectrometer (LCMS-2020, Shimadzu, Japan) equipped with an ESI source. The chromatographic separation was conducted using a YMC Pack Pro column, 3 × 150 mm (YMC, Japan) at 40°C and a mobile phase that consisted of 0.1% formic acid water solution (solvent A), and acetonitrile (solvent B). Mass spectrometry data were acquired in both positive and negative ionization mode, and analyzed using the LabSolutions LCMS software.

General procedure for the synthesis of acyclic nucleoside diesters 1–5

A solution of heterocyclic base (2.5 mmol), dimethyl itaconate (592 mg, 3.75 mmol), and 1,8-diazabicyclo[5.4.0]undec-7-ene (373 µL, 2.5 mmol) was stirred in 2.5 mL

of *N,N*-dimethylformamide at room temperature for 24 h. After the removal of solvent under reduced pressure, the residue was dissolved in ethyl acetate (50 mL) and washed with water. Acyclic nucleoside diesters **1, 3**, and **4** with halogen and hydrogen atoms at the 4th position of heterocyclic base were dried over anhydrous sodium sulphate, concentrated and purified by column chromatography (silica gel, chloroform/methanol, 98/2). Acyclic nucleoside diesters with hydroxyl and carboxyl groups (**2** and **5**) were purified by reverse phase chromatography (C-18 cartridges, methanol/water mixture, 10:0→10:3). After purification, solvents were removed under reduced pressure to afford white colorless solid reaction products **1**–**5**.

Dimethyl 2-((2-oxo-1-pyridyl)methyl)butanedioate (1). Yield 398 mg (63%), white solid. UV λ_{max}: 304 nm; MS (ESI^+): *m/z* 254.00 $[M+H]^+$. ^1H NMR ($CDCl_3$, ppm): $\delta = 2.72–2.74$ (m, 2H, CH_2), 3.38–3.45 (m, 1H, CH), 3.69 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 4.19–4.23 (m, 2H, CH_2), 6.17 (td, $J = 6.7$, 1.4 Hz, 1H, CH), 6.57–6.60 (m, 1H, CH), 7.30–7.38 (m, 2H, CH); ^{13}C NMR ($CDCl_3$, ppm): $\delta = 33.51$ (CH_2), 40.29 (CH), 50.81 (CH_2), 51.95 (CH_3), 52.28 (CH_3), 106.00 (CH), 120.94 (CH), 138.34 (CH), 139.87 (CH), 162.69 (C=O), 171.63 (C=O), 173.0 (C=O).

Dimethyl 2-((4-hydroxy-2-oxo-1-pyridyl)methyl)butanedioate (2). Yield 531 mg (79%), colorless powder. UV λ_{max}: 283 nm; MS (ESI^+): *m/z* 270.00 $[M+H]^+$, 268.00 $[M-H]^-$. ^1H NMR (DMSO-d_6, ppm): $\delta = 3.19–3.24$ (m, 2H, CH_2), 3.38–3.45 (m, 1H, CH), 3.56 (s, 3H, CH_3), 3.57 (s, 3H, CH_3), 3.77–3.91 (m, 2H, CH_2), 5.01 (d, $J = 2.4$ Hz, 1H, CH), 5.47 (dd, $J = 7.5$, 2.5 Hz, 1H, CH), 7.00 (d, $J = 7.6$ Hz, 1H, CH); ^{13}C NMR (DMSO-d_6, ppm): $\delta = 33.14$ (CH_2), 41.37 (CH), 48.39 (CH_2), 51.97 (CH_3), 52.20 (CH_3), 96.91 (CH), 113.23 (CH), 136.91 (CH), 164.22 (C-OH), 164.79 (C=O), 172.01 (C=O), 173.42 (C=O). In agreement with the literature data.[11]

Dimethyl 2-((4-chloro-2-oxo-1-pyridyl)methyl)butanedioate (3). Yield 416 mg (58%), white solid. UV λ_{max}: 306 nm; MS (ESI^+): *m/z* 288.05/290.05 $[M+H]^+$. ^1H NMR ($CDCl_3$, ppm): $\delta = 2.73$ (dd, $J = 6.1$, 4.2 Hz, 2H, CH_2), 3.32–3.39 (m, 1H, CH), 3.69 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 4.18 (d, $J = 6.7$ Hz, 2H, CH_2), 6.20 (dd, $J = 7.3$, 2.2 Hz, 1H, CH), 6.61 (d, $J = 2.2$ Hz, 1H, CH), 7.32 (d, $J = 7.3$ Hz, 1H, CH); ^{13}C NMR ($CDCl_3$, ppm): $\delta = 33.57$ (CH_2), 40.17 (CH), 50.54 (CH_2), 52.01 (CH_3), 52.36 (CH_3), 107.79 (CH), 119.30 (CH), 138.57 (CH), 147.13 (CCl), 161.60 (C=O), 171.51 (C=O), 172.78 (C=O).

Dimethyl 2-((4-bromo-2-oxo-1-pyridyl)methyl)butanedioate (4). Yield 547 mg (66%), white solid. UV λ_{max}: 307 nm; MS (ESI^+): *m/z* 332.00/334.00 $[M+H]^+$. ^1H NMR (DMSO-d_6, ppm): $\delta = 2.58–2.63$ (m, 2H, CH_2), 3.19–3.26 (m, 1H, CH), 3.56 (s, 3H, CH_3), 3.57 (s, 3H, CH_3), 4.00–4.15 (m, 2H, CH_2), 6.47 (dd, $J = 7.3$, 2.2 Hz, 1H, CH), 6.73 (d, $J = 2.2$ Hz, 1H, CH), 7.60 (d, $J = 7.3$ Hz, 1H, CH); ^{13}C NMR (DMSO-d_6, ppm): $\delta = 33.29$ (CH_2), 40.4 (CH), 49.74 (CH_2), 52.07 (CH_3), 52.41

(CH_3), 109.61 (CH), 121.77 (CH), 135.70 (CH), 140.39 (CBr), 160.91 (C=O), 171.72 (C=O), 172.73 (C=O).

1-(4-methoxy-2-methoxycarbonyl-4-oxo-butyl)-6-oxo-pyridine-3-carboxylic acid (5). Yield 578 mg (78%), colorless powder. UV λ_{max}: 250, 295 nm; MS (ESI^+): *m/z* 298.00 $[M+H]^+$, 296.00 $[M-H]^-$. ^1H NMR (DMSO-d_6, ppm): δ = 2.86–2.92 (m, 2H, CH_2), 3.29 (s, 3H, CH_3), 3.31 (s, 3H, CH_3), 3.32–3.34 (m, 1H, CH), 3.99–4.04 (m, 2H, CH_2), 6.26 (d, J = 9.2 Hz, 1H, CH), 7.85 (dd, J = 9.2, 2.4 Hz, 1H, CH), 8.06 (d, J = 2.3 Hz, 1H, CH); ^{13}C NMR (DMSO-d_6, ppm): δ = 32.71 (CH_2), 35.84 (CH), 48.13 (CH_2), 59.16 (CH_3), 59.33 (CH_3), 117.28 (CCOOH), 124.35 (CH), 133.35 (CH), 141.57 (CH), 162.70 (C=O), 167.17 (C=O), 167.47 (C=O), 168.22 (C=O).

Reduction of acyclic nucleosides

Calcium chloride ($CaCl_2$) (333 mg, 3 mmol) and $NaBH_4$ (227 mg, 6 mmol) were stirred in 5 mL of anhydrous tetrahydrofuran (THF) at 0°C for 1 h. A suspension of synthesized acyclic nucleoside diester (1.5 mmol) in anhydrous THF (5 mL) was added, and the reaction mixture was stirred at room temperature overnight. After adding of methanol (1 mL), the solvents were evaporated under reduced pressure. The crude light brown residue was taken up with 10 mL of ethanol, insoluble $CaCl_2$ was filtered, and the solvent was evaporated under reduced pressure. The residue was dissolved in water (2 mL) and purified by reverse phase column chromatography (C-18 cartridges, methanol/water mixture, 10:0→10:2). The solvents were removed under reduced pressure to afford colorless or yellowish oil reaction products **6**–**10**.

1-(4-hydroxy-2-hydroxymethyl-butyl)pyridin-2-one (6). Yield 216 mg (73%), yellowish oil; R_f = 0.66 (chloroform/methanol, 4:1). UV λ_{max}: 305 nm; MS (ESI^+): *m/z* 198.10 $[M+H]^+$. ^1H NMR (DMSO-d_6, ppm): δ = 1.31–1.40 (m, 1H, CH_2), 1.45–1.54 (m, 1H, CH_2), 1.93–2.01 (m, 1H, CH), 3.24–3.31 (m, 2H, CH_2), 3.39–3.49 (m, 2H, CH_2), 3.79–3.91 (m, 2H, CH_2), 4.46 (s, 1H, OH), 4.62 (s, 1H, OH), 6.22 (td, J = 6.7, 1.4 Hz, 1H, CH), 6.39 (ddd, J = 9.1, 1.3, 0.6 Hz, 1H, CH), 7.39–7.44 (m, 1H, CH), 7.61 (ddd, J = 6.7, 2.1, 0.6 Hz, 1H, CH); ^{13}C NMR (DMSO-d_6, ppm): δ = 31.91 (CH_2), 37.72 (CH), 50.57 (CH_2), 59.14 (CH_2), 60.99 (CH_2), 105.60 (CH), 120.20 (CH), 140.24 (CH), 140.33 (CH), 162.35 (C=O).

4-hydroxy-1-(4-hydroxy-2-hydroxymethyl-butyl)pyridin-2-one (7). Yield 265 mg (83%), yellowish oil; R_f = 0.51 (chloroform/methanol, 4:1). UV λ_{max}: 280 nm; MS (ESI^+): *m/z* 214.05 $[M+H]^+$, 212.05 $[M-H]^-$. ^1H NMR (DMSO-d_6): δ = 1.31–1.45 (m, 2H, CH_2), 1.67–1.73 (m, 1H, CH), 3.11 (bs, 1H, OH), 3.16 (bs, 1H, OH), 3.43–3.52 (m, 4H, $2CH_2$), 3.51–3.73 (m, 2H, CH_2), 4.80 (d, J = 2.5 Hz, 1H, CH), 5.33 (dd, J = 7.5, 2.5 Hz, 1H, CH), 5.66 (s, 1H, OH), 6.91 (d, J = 7.5 Hz, 1H, CH); ^{13}C NMR

(DMSO-d_6): δ = 32.48 (CH_2), 39.21 (CH), 47.09 (CH_2), 59.32 (CH_2), 60.87 (CH_2), 97.26 (CH), 110.67 (CH), 135.40 (CH), 139.58 (COH), 165.41 (C=O). In agreement with the literature data.[11]

4-chloro-1-(4-hydroxy-2-hydroxymethyl-butyl)pyridin-2-one (8). Yield 80 mg (23%), colorless oil; R_f = 0.75 (chloroform/methanol, 4:1). UV λ_{max}: 304 nm; MS (ESI^+): *m/z* 232.10/234.10 $[M+H]^+$. ^1H NMR (DMSO-d_6, ppm): δ = 1.28–1.37 (m, 1H, CH_2), 1.42–1.53 (m, 1H, CH_2), 1.93–2.01 (m, 1H, CH), 3.29–3.32 (m, 2H, CH_2), 3.39–3.47 (m, 2H, CH_2), 3.79–3.90 (m, 2H, CH_2), 4.44 (s, 1H, OH), 4.61 (s, 1H, OH), 6.37 (dd, *J* = 7.3, 2.4 Hz, 1H, CH), 6.54 (d, *J* = 2.3 Hz, 1H, CH), 7.70 (d, *J* = 7.3 Hz, 1H, CH); ^{13}C NMR (DMSO-d_6, ppm): δ = 31.69 (CH_2), 36.92 (CH), 50.66 (CH_2), 59.06 (CH_2), 60.68 (CH_2), 106.76 (CH), 118.17 (CH), 141.16 (CH), 145.86 (CCl), 161.32 (C=O).

4-bromo-1-(4-hydroxy-2-hydroxymethyl-butyl)pyridin-2-one (9). Yield 112 mg (27%), colorless oil; R_f = 0.77 (chloroform/methanol, 4:1). UV λ_{max}: 310 nm; MS (ESI^+): *m/z* 275.95/277.95 $[M+H]^+$. ^1H NMR (DMSO-d_6, ppm): δ = 1.27–1.37 (m, 1H, CH_2), 1.43–1.52 (m, 1H, CH_2), 1.92–2.01 (m, 1H, CH), 3.26–3.32 (m, 2H, CH_2), 3.38–3.48 (m, 2H, CH_2), 3.77–3.89 (m, 2H, CH_2), 4.46 (s, 1H, OH), 4.61 (s, 1H, OH), 6.47 (dd, *J* = 7.2, 2.2 Hz, 1H, CH), 6.71 (d, *J* = 2.2 Hz, 1H, CH), 7.61 (d, *J* = 7.2 Hz, 1H, CH); ^{13}C NMR (DMSO-d_6): δ = 32.12 (CH_2), 37.10 (CH), 50.70 (CH_2), 59.05 (CH_2), 61.04 (CH_2), 109.29 (CH), 121.64 (CH), 134.95 (CH), 140.83 (CBr), 161.15 (C=O).

1-(4-hydroxy-2-hydroxymethyl-butyl)-6-oxo-pyridine-3-carboxylic acid (10). Yield 148 mg (41%), yellowish oil; R_f = 0.78 (1,4-dioxane/water/2-propanol/ammonia water, 4:2:2:1). UV λ_{max}: 255, 295 nm; MS (ESI^+): *m/z* 242.05 $[M+H]^+$, 240.05 $[M-H]^-$. ^1H NMR (DMSO-d_6, ppm): δ = 1.27–1.54 (m, 2H, CH_2), 1.94–2.00 (m, 1H, CH), 3.28–3.32 (m, 2H, CH_2), 3.86–3.90 (m, 2H, CH_2), 4.00–4.06 (m, 2H, CH_2), 4.34 (s, 1H, OH), 4.46 (s, 1H, OH), 6.30 (d, *J* = 9.3 Hz, 1H, CH), 7.83 (dd, *J* = 9.3, 2.4 Hz, 1H, CH), 8.10 (d, *J* = 2.3 Hz, 1H, CH); ^{13}C NMR (DMSO-d_6, ppm): δ = 32.66 (CH_2), 35.75 (CH), 49.77 (CH_2), 60.81 (CH_2), 61.71 (CH_2), 117.34 (CCOOH), 120.49 (CH), 138.63 (CH), 141.01 (CH), 162.66 (C=O), 167.17 (C=O).

General procedure for the synthesis of protected nucleosides 11–15

To a suspension of an appropriate heterocyclic base (1 mmol) in 2 mL of acetonitrile, 243 µL (1 mmol) of BSA was added. In the case of synthesis of compounds **12** and **15**, 2 mmol of BSA were added. The reaction mixture was stirred at room temperature for 40 min, and 260 mg (1 mmol) of 1,3,5-tri-O-acetyl-D-ribose was dissolved in 0.5 mL of acetonitrile, and 117 µL (1 mmol) of $SnCl_4$ was added. The reaction mixture was stirred at room temperature overnight. The reaction mixture of protected

nucleosides with halogen and hydrogen atoms at the 4th position of heterocyclic base (**11**, **13**, and **14**) was dissolved in 60 mL of ethyl acetate and washed for five times with saturated $NaHCO_3$ solution. The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, chloroform/methanol mixture, 10:0→10:0.5). The crude reaction mixture of protected nucleosides with hydroxyl and carboxyl groups of heterocyclic base (**12** and **15**) was concentrated under reduced pressure and dissolved in 2 mL of methanol/water, 3/1 solution. The precipitate was filtered and washed twice with 1 mL of methanol/water, 3/1 solution. The filtrate was concentrated under reduced pressure, the residue was dissolved in water (1 mL) and purified by reverse phase column chromatography (C-18 cartridges, methanol/water mixture, 10:0→10:4). The solvents were removed under reduced pressure to afford colorless oil reaction products **11**–**15**.

(3-acetoxy-5-(2-oxo-1-pyridyl)tetrahydrofuran-2-yl)methyl acetate (11). Yield 286 mg (97%), colorless oil. UV λ_{max}: 306 nm; MS (ESI^+): *m*/*z* 296.00 $[M+H]^+$. ^1H NMR (DMSO-d_6, ppm): δ = 2.08 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.24–2.32 (m, 1H, CH_2), 2.78–2.91 (m, 1H, CH_2), 3.98–4.03 (m, 1H, CH_2), 4.13–4.18 (m, 1H, CH_2), 4.79 (t, *J* = 4.4 Hz, 1H, CH), 5.11–5.16 (m, 1H, CH), 5.12–5.24 (m, 1H, CH), 6.24–6.43 (m, 2H, CH), 7.44–7.47 (m, 1H, CH), 7.72–7.78 (m, 1H, CH); ^{13}C NMR (DMSO-d_6, ppm): δ = 21.03 (CH_3), 21.24 (CH_3), 37.68 (CH_2), 63.69 (CH_2), 74.68 (CH), 82.24 (CH), 87.52 (CH), 106.30 (CH), 120.11 (CH), 133.92 (CH), 140.86 (CH), 161.49 (C=O), 169.70 (C=O), 170.36 (C=O).

(3-acetoxy-5-(4-hydroxy-2-oxo-1-pyridyl)tetrahydrofuran-2-yl)methyl acetate (12). Yield 255 mg (82%), colorless oil. UV λ_{max}: 283 nm; MS (ESI^+): *m*/*z* 312.00 $[M+H]^+$, 310.00 $[M-H]^-$. ^1H NMR (DMSO-d_6): δ = 2.06 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.14–2.27 (m, 1H, CH_2), 2.65–2.78 (m, 1H, CH_2), 3.81 (s, 1H, OH), 4.07–4.22 (m, 2H, CH_2), 4.47–4.52 (m, 1H, CH), 5.01–5.21 (m, 1H, CH), 5.42–5.59 (m, 1H, CH), 6.29–6.51 (m, 2H, CH), 6.97–7.21 (m, 2H, CH); ^{13}C NMR (DMSO-d_6): δ = 21.08 (CH_3), 21.27 (CH_3), 37.99 (CH_2), 63.50 (CH_2), 74.91 (CH), 82.93 (CH), 95.73 (CH), 96.69 (CH), 108.30 (CH), 120.57 (CH), 130.36 (CH), 164.02 (C=O), 169.87 (C=O), 170.65 (C=O).

(3-acetoxy-5-(4-chloro-2-oxo-1-pyridyl)tetrahydrofuran-2-yl)methyl acetate (13). Yield 277 mg (84%), colorless oil. UV λ_{max}: 306 nm; MS (ESI^+): *m*/*z* 330.00/332.00 $[M+H]^+$. ^1H NMR (DMSO-d_6, ppm): δ = 2.04 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.23–2.34 (m, 1H, CH_2), 2.79–2.88 (m, 1H, CH_2), 3.98–4.03 (m, 1H, CH_2), 4.23–4.32 (m, 1H, CH_2), 4.81 (t, *J* = 4.7 Hz, 1H, CH), 5.07–5.15 (m, 1H, CH), 5.18–5.22 (m, 1H, CH), 6.40 (dd, *J* = 7.6, 2.3 Hz, 1H, CH), 6.53 (d, *J* = 2.3 Hz, 1H, CH), 7.83 (d, *J* = 7.6 Hz, 1H, CH). ^{13}C NMR (DMSO-d_6): δ = 20.68 (CH_3), 21.27

(CH_3), 36.89 (CH_2), 62.58 (CH_2), 73.91 (CH), 83.23 (CH), 93.73 (CH), 116.34 (CH), 121.30 (CH), 131.57 (CH), 141.36 (CCl), 160.02 (C=O), 169.87 (C=O), 170.65 (C=O).

(3-acetoxy-5-(4-bromo-2-oxo-1-pyridyl)tetrahydrofuran-2-yl)methyl acetate (14). Yield 322 mg (86%), colorless oil. UV λ_{max}: 306 nm; MS (ESI^+): *m/z* 373.95/375.95 $[M+H]^+$. ^1H NMR (DMSO-d_6, ppm): δ = 2.05 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.23–2.35 (m, 1H, CH_2), 2.76–2.89 (m, 1H, CH_2), 3.99–4.03 (m, 1H, CH_2), 4.24–4.29 (m, 1H, CH_2), 4.80 (t, *J* = 4.7 Hz, 1H, CH), 5.08–5.15 (m, 1H, CH), 5.18–5.22 (m, 1H, CH), 6.51 (dd, *J* = 7.5, 2.1 Hz, 1H, CH), 6.70 (d, *J* = 2.1 Hz, 1H, CH), 7.74 (d, *J* = 7.5, Hz, 1H, CH). ^{13}C NMR (DMSO-d_6): δ = 20.56 (CH_3), 21.29 (CH_3), 35.90 (CH_2), 61.84 (CH_2), 73.68 (CH), 83.25 (CH), 92.73 (CH), 116.34 (CH), 119.87 (CH), 137.20 (CH), 139.04 (CBr), 159.02 (C=O), 170.17 (C=O), 171.45 (C=O).

1-(4-acetoxy-5-(acetoxymethyl)tetrahydrofuran-2-yl)-6-oxo-pyridine-3-carboxylic acid (15). Yield 108 mg (32%), colorless oil. UV λ_{max}: 259, 294 nm; MS (ESI^+): *m/z* 340.00 $[M+H]^+$, 338.00 $[M-H]^-$. ^1H NMR (DMSO-d_6, ppm): δ = 2.07 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.26–2.37 (m, 1H, CH_2), 2.76–2.89 (m, 1H, CH_2), 4.07–4.17 (m, 2H, CH_2), 4.74 (t, *J* = 4.9 Hz, 1H, CH), 5.21–5.25 (m, 1H, CH), 6.32–6.36 (m, 1H, CH), 6.43 (d, *J* = 7.2 Hz, 1H, CH), 7.83 (dd, *J* = 7.2, 2.2 Hz, 1H, CH), 8.41 (d, *J* = 2.2, Hz, 1H, CH), 12.97 (bs, 1H, OH). ^{13}C NMR (DMSO-d_6): δ = 20.65 (CH_3), 21.32 (CH_3), 35.80 (CH_2), 61.81 (CH_2), 73.59 (CH), 83.20 (CH), 90.23 (CH), 116.84 (CCOOH), 119.90 (CH), 135.70 (CH), 142.88 (CH), 161.02 (C=O), 167.24 (C=O), 170.17 (C=O), 171.45 (C=O).

Deprotection of modified nucleosides

Synthesized protected nucleoside (0.5 mmol) was dissolved in 5 mL of methanol, and 1 mL of 1 M sodium methylate solution was added dropwise. Reaction mixture was stirred at room temperature for 20 min. Completion of the reaction was determined by TLC. After neutralization with 10% acetic acid solution, the solvent was removed under reduced pressure, and the residue was purified by reverse phase chromatography (C-18 cartridges, methanol/water mixture, 10:0→10:2). The solvents were removed under reduced pressure to afford colorless oil reaction products **16**–**20**.

1-(4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyridin-2-one (16). Yield 77 mg (73%), colorless oil. R_f = 0.55 (chloroform/methanol, 4:1). UV λ_{max}: 300 nm; MS (ESI^+): *m/z* 212.00 $[M+H]^+$. ^1H NMR (DMSO-d_6, ppm): δ = 2.16–2.33 (m, 1H, CH_2), 2.53–2.75 (m, 1H, CH_2), 3.42–3.47 (m, 1H, CH_2), 3.58–3.61 (m, 1H, CH_2), 3.83–3.87 (m, 1H, CH), 4.24 (m, 1H, CH), 4.74 (bs, 1H, OH), 4.90 (bs, 1H, OH), 5.83 (m, 1H, CH), 6.32–6.41 (m, 2H, CH), 7.40–7.42

(m, 1H, CH), 7.93–7.96 (m, 1H, CH); ^{13}C NMR (DMSO-d_6, ppm): δ = 41.48 (CH_2), 62.22 (CH_2), 71.13 (CH), 86.93 (CH), 90.27 (CH), 105.17 (CH), 119.69 (CH), 133.96 (CH), 140.26 (CH), 161.53 (C=O). In agreement with the literature data.[13]

4-hydroxy-1-(4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyridin-2-one (17). Yield 82 mg (72%), colorless oil. R_f = 0.37 (chloroform/methanol, 4:1). UV λ_{max}: 281 nm; MS (ESI$^+$): *m/z* 228.05 [M+H]$^+$, 226.00 [M-H]$^-$. ^{1}H NMR (DMSO-d_6, ppm): δ = 1.86–2.00 (m, 1H, CH_2), 2.32–2.36 (m, 1H, CH_2), 3.32–3.43 (m, 1H, CH_2), 3.46–3.55 (m, 1H, CH_2), 3.63–3.74 (m, 1H, CH), 4.08–4.24 (m, 1H, CH), 4.75 (s, 1H, OH), 4.84 (s, 1H, OH), 5.35–5.43 (m, 1H, CH), 6.23 (dd, *J* = 7.6, 2.9 Hz, 1H, CH), 7.01–7.29 (m, 2H, CH); ^{13}C NMR (DMSO-d_6, ppm): δ = 40.92 (CH_2), 61.61 (CH_2), 71.41 (CH), 86.91 (CH), 88.13 (CH), 96.81 (CH), 108.68 (CH), 131.74 (CH), 132.72 (COH), 164.09 (C=O). The ^{1}H NMR spectrum is consistent with the previously reported one.[19]

4-chloro-1-(4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyridin-2-one (18). Yield 80 mg (65%), colorless oil. R_f = 0.63 (chloroform/methanol, 4:1). UV λ_{max}: 306 nm; MS (ESI$^+$): *m/z* 246.05/248.05 [M+H]$^+$. ^{1}H NMR (DMSO-d_6, ppm): δ = 2.22–2.34 (m, 1H, CH_2), 2.54–2.66 (m, 1H, CH_2), 3.42–3.45 (m, 1H, CH_2), 3.55–3.63 (m, 1H, CH_2), 4.28 (t, *J* = 4.7 Hz, 1H, CH), 4.86–4.91 (m, 1H, CH), 5.07 (t, *J* = 5.2 Hz, 1H, OH), 5.27 (d, *J* = 4.2 Hz, 1H, OH), 5.72–5.80 (m, 1H, CH), 6.41 (dd, *J* = 7.6, 2.3 Hz, 1H, CH), 6.48 (dd, *J* = 2.3 Hz, 1H, CH), 7.87 (d, *J* = 7.6 Hz, 1H, CH). ^{13}C NMR (DMSO-d_6): δ = 38.70 (CH_2), 61.98 (CH_2), 72.96 (CH), 84.23 (CH), 93.21 (CH), 116.34 (CH), 123.67 (CH), 131.17 (CH), 141.96 (CCl), 159.82 (C=O).

4-bromo-1-(4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyridin-2-one (19). Yield 91 mg (63%), colorless oil. R_f = 0.67 (chloroform/methanol, 4:1). UV λ_{max}: 304 nm; MS (ESI$^+$): *m/z* 289.95/291.95 [M+H]$^+$. ^{1}H NMR (DMSO-d_6, ppm): δ = 2.25–2.32 (m, 1H, CH_2), 2.54–2.65 (m, 1H, CH_2), 3.41–3.44 (m, 1H, CH_2), 3.57–3.69 (m, 1H, CH_2), 3.83–3.89 (m, 1H, CH), 4.25–4.29 (m, 1H, CH), 4.92 (bs, 1H, OH), 5.16 (bs, 1H, OH), 6.22–6.28 (m, 1H, CH), 6.51 (dd, *J* = 7.5, 2.2 Hz, 1H, CH), 6.66 (dd, *J* = 2.2 Hz, 1H, CH), 7.78 (m, *J* = 7.5 Hz, 1H, CH). ^{13}C NMR (DMSO-d_6): δ = 36.90 (CH_2), 61.84 (CH_2), 72.20 (CH), 84.29 (CH), 91.23 (CH), 117.34 (CH), 119.89 (CH), 137.80 (CH), 139.55 (CBr), 158.60 (C=O).

1-(4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-6-oxo-pyridine-3-carboxylic acid (20). Yield 92 mg (72%), colorless oil. R_f = 0.71 (1,4-dioxane/water/2-propanol/ammonia water, 4:5:2:1). UV λ_{max}: 256, 295 nm; MS (ESI$^+$): *m/z* 256.05 [M+H]$^+$, 254.00 [M-H]$^-$. ^{1}H NMR (DMSO-d_6,

ppm): δ = 1.89–1.99 (m, 1H, CH_2), 2.26–2.33 (m, 1H, CH_2), 3.44–3.48 (m, 1H, CH_2), 3.58–3.66 (m, 1H, CH_2), 3.69–3.83 (m, 1H, CH), 4.21–4.30 (m, 1H, CH), 4.76 (bs, 2H, OH), 6.13–6.19 (m, 1H, CH), 6.24–6.39 (m, 1H, CH), 7.82–7.86 (m, 1H, CH), 8.37–8.47 (m, 1H, CH). ^{13}C NMR (DMSO-d_6): δ = 36.96 (CH_2), 61.89 (CH_2), 72.89 (CH), 84.70 (CH), 89.68 (CH), 116.56 (CCOOH), 119.40 (CH), 134.98 (CH), 142.30 (CH), 160.80 (C=O), 167.44 (C=O).

General procedure for the synthesis of nucleotides 21–25

To a suspension of synthesized nucleoside (0.25 mmol), 54 mg (0.25 mmol) "proton sponge" in 1.5 mL of triethyl phosphate cooled to 0°C, 47 µL (0.5 mmol) of phosphorous oxychloride was added and the reaction mixture was stirred at room temperature for 80 min. After the reaction was completed (TLC), 43 µL (18 mmol) of tributylamine and 2.5 mL of 0.5 M tributylammonium pyrophosphate solution in DMF were added dropwise. After 7 min of stirring, the reaction mixture was poured into ice water and neutralized with saturated sodium bicarbonate solution. The reaction mixture was purified by ion exchange chromatography on DEAE-Sephadex A25 columns (20 mL) with a linear gradient (0.05–0.3 M) of LiCl as the mobile phase. The product was eluted with 0.28–0.3 M LiCl, the solution was concentrated under reduced preassure to several milliliters and poured into a 40-mL mixture of acetone/methanol, 4/1. The formed precipitate was collected by centrifugation (4000 rpm, 10 min) and washed twice with a mixture of acetone/methanol, 4/1. The nucleotide was dissolved in 2 mL of water and evaporated under reduced pressure. Slightly acidic solution of nucleotide was neutralized with 1 M sodium hydroxide solution to pH 7.0.

(3-hydroxy-5-(2-oxo-1-pyridyl)tetrahydrofuran-2-yl)methyl triphosphate (21). Yield 4.4 mg (3.9%). R_f = 0.14 (1,4-dioxane/water/2-propanol/ammonia water, 4:5:2:1). UV λ_{max}: 298 nm; MS (ESI$^+$): *m/z* 451.80 $[M+H]^+$, 449.75 $[M-H]^-$. ^{1}H NMR (D_2O, ppm): δ = 2.17–2.25 (m, 1H, CH_2), 2.40–2.48 (m, 1H, CH_2), 3.99–4.02 (m, 2H, CH_2), 4.14– 4.17 (m, 1H, CH), 4.46 4.49 (m, 1H, CH), 6.25 (dd, J = 7.1 Hz, 2.2 Hz, 1H, CH), 6.42 (t, J = 6.4 Hz, 1H, CH), 6.49 (m, 1H, CH), 7.56 (m, 1H, CH), 7.88 (d, J = 7.1 Hz, 1H, CH). ^{31}P NMR (D_2O, ppm): δ = −20.10 (t, P_β), −10.73 (d, P_α), −6.61 (d, P_γ). The ^{31}P NMR spectrum was consistent with the one reported previously.[13]

(3-hydroxy-5-(4-hydroxy-2-oxo-1-pyridyl)tetrahydrofuran-2-yl)methyl triphosphate (22). Yield 3.3%. R_f = 0.11 (1,4-dioxane/water/2-propanol/ammonia water, 4:5:2:1). UV λ_{max}: 275 nm; MS (ESI$^+$): *m/z* 468.05 $[M+H]^+$, 469.00 $[M-H]^-$. ^{1}H NMR (D_2O, ppm): δ = 2.00–2.03 (m, 1H, CH_2), 2.10–2.13 (m, 1H, CH_2), 3.95–4.13 (m, 2H, 1H, CH_2, CH), 4.44–4.49 (m, 1H, CH), 5.95–5.99 (m, 1H, CH), 6.19–6.24 (m, 1H, CH), 6.38–6.43 (m, 1H, CH), 7.59–7.63 (m, 1H, CH). ^{31}P NMR (D_2O, ppm): δ = −20.23 (t, P_β), −10.74 (d, P_α), −6.64 (d, P_γ).

(5-(4-chloro-2-oxo-1-pyridyl)-3-hydroxytetrahydrofuran-2-yl)methyl triphosphate (23). Yield 6.1%. R_f = 0.18 (1,4-dioxane/water/2-propanol/ammonia water, 4:5:2:1). UV λ_{max}: 302 nm; MS (ESI^+): *m/z* 485.75/487.80 $[M+H]^+$, 483.70/485.70 $[M-H]^-$. ^1H NMR (D_2O, ppm): δ = 2.17–2.24 (m, 1H, CH_2), 2.39–2.47 (m, 1H, CH_2), 3.98–4.16 (m, 2H, 1H, CH_2, CH), 4.44–4.48 (m, 1H, CH), 6.19 (dd, J = 7.3, 2.0 Hz, 1H, CH), 6.32 (t, J = 6.4 Hz, 1H, CH), 6.57 (m, 1H, CH), 7.84 (d, 1H, CH, J = 7.3 Hz). ^{31}P NMR (D_2O, ppm): δ = −20.87 (t, P_β), −10.78 (d, P_α), −6.24 (d, P_γ).

(5-(4-bromo-2-oxo-1-pyridyl)-3-hydroxytetrahydrofuran-2-yl)methyl triphosphate (24). Yield 10.2%. R_f = 0.17 (1,4-dioxane/water/2-propanol/ammonia water, 4:5:2:1). UV λ_{max}: 301 nm; MS (ESI^+): *m/z* 529.75/531.75 $[M+H]^+$, 527.65/529.70 $[M-H]^-$. ^1H NMR (D_2O, ppm): δ = 2.16–2.25 (m, 1H, CH_2), 2.39–2.47 (m, 1H, CH_2), 3.96–4.16 (m, 2H, 1H, CH_2, CH), 4.45–4.48 (m, 1H, CH), 6.16 (dd, J = 7.1, 2.1 Hz, 1H, CH), 6.30 (t, J = 6.2 Hz, 1H, CH), 6.67 (m, 1H, CH), 7.76 (d, J = 7.1 Hz, 1H, CH). ^{31}P NMR (D_2O, ppm): δ = −20.94 (t, P_β), −10.80 (d, P_α), −6.09 (d, P_γ).

(5-(5-carboxy-2-oxo-1-pyridyl)-3-hydroxytetrahydrofuran-2-yl)methyl triphosphate (25). Yield 5.2%. R_f = 0.10 (1,4-dioxane/water/2-propanol/ammonia water, 4:5:2:1). UV λ_{max}: 252, 298 nm; MS (ESI^+): *m/z* 493.75 $[M-H]^-$. ^1H NMR (D_2O, ppm): δ = 1.96–2.04 (m, 1H, CH_2), 2.27–236 (m, 1H, CH_2), 3.54–3.70 (m, 2H, 1H, CH_2, CH), 4.02–4.05 (m, 1H, CH), 5.90 (t, J = 6.7 Hz, 1H, CH), 6.25–6.39 (m, 1H, C=CH), 6.96–7.04 (m, 1H, C=CH), 8.26–8.37 (m, 1H, C=CH). ^{31}P NMR (D_2O, ppm): δ = −20.26 (t, P_β), −10.14 (d, P_α), −5.98 (d, P_γ).

DNA biosynthesis assay

For testing of the inhibitory properties of nucleosides, reactions were performed as described[20] with modifications using duplex DNA comprising 5′ ^{33}P-labelled primer oligodeoxyribonucleotide 5′-TAATACGACTCACTATAGGGAGA and template oligodeoxyribonucleotide 5′-CCGGAATTAAAATCTCCCTATAGTG AGTCGTATTA, annealed by heating for 5 min at 95°C and gradually cooling down to room temperature over 2 h. 5 nM DNA duplex was subjected to primer extension reactions in the presence of 50 nM polymerases Klenow exo-, M.MuLV, and HIV-1 and 100 nM dTTP in a reaction mix comprising 20 mM sodium glutamate, pH 8.2, 10 mM DTT, 0.5% Triton X-100, 20 mM sodium chloride, and 1 mM magnesium chloride. The reactions were performed for 5 min at 37°C in the presence of 1–1000 μM compounds **6**–**10** and **16**–**20**. The reactions were terminated by adding an equal volume of STOP solution, containing 95% deionised formamide and 100 mm EDTA. The products were resolved on a 15% 29:1 denaturing (7 M urea) polyacrylamide gel, the gel was dried on Whatman paper and autoradiographed using a phosphorimager screen.

For the acceptance of nucleotide triphosphates for DNA biosynthesis, four different template oligodeoxyribonucleotides were employed for DNA duplex formation

(residues governing nature of first and subsequent nucleotides to be incorporated are in **bold**):

5′-CCGGAATT**AAAA**TCTCCCTATAGTGAGTCGTATTA (Template "A");
5′-TTAAGGCC**GGGG**TCTCCCTATAGTGAGTCGTATTA (Template "G");
5′-CCGGTTAA**TTTT**TCTCCCTATAGTGAGTCGTATTA (Template "T");
5′-TTAACCGG**CCCC**TCTCCCTATAGTGAGTCGTATTA (Template "C").

Primer extension was performed using either 1 μM or 10 μM compounds **21**–**25** under the same conditions as described above, dTTP was omitted. Reaction products were separated, and gel was processed as described.

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